DATA EVALUATION RECORD

***Beauveria bassiana* strain 447**

STUDY TYPE: ACUTE ORAL TOXICITY/PATHOGENICITY - RAT (885.3050)
MRID 45144201

Prepared for
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U.S. Environmental Protection Agency
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DATA EVALUATION REPORT

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STUDY TYPE:	Acute Oral Toxicity and Pathogenicity - Rats (OPPTS 885.3050)
MRID NO:	45144201
CHEMICAL CODE	128815
EPA REGISTRATION#	070464-U
SUBMISSION #	S585216
DATA PACKAGE #	268975
TEST MATERIAL:	*Beauveria bassiana* 447
PROJECT NO:	1179 SN3
SPONSOR:	SafeScience, Inc., Boston, MA
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Toxicity/Pathogenicity Testing of *Beauveria bassiana* following Acute Oral Challenge in Rats
AUTHOR(S):	William M. Mega, B.S.
STUDY COMPLETED:	May 2000
GOOD LABORATORY PRACTICE:	GLP Compliant except for test substance characterization
CONCLUSION:	*Beauveria bassiana,* isolate 447 does not appear to be toxic and/or pathogenic in rats, when dosed by oral gavage at 5.1×10^7 cfu/animal.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: Microbial pest control agent (MPCA), *Beauveria bassiana,* isolate 447 (approximately 5.1×10^7 cfu/mL)

Test Animals: Fifty-one male and 51 female CD® rats were received from Charles River Laboratories, Inc., Raleigh, NC. Thirty-nine males and 39 females, approximately 51 days old, were assigned to groups, and weighed in at 175-212 g (males) and 140-168 g (females) on the day of dosing. The rats were housed two per plastic cage. Tap water and food (Certified Purina Rodent Chow 5002) were provided *ad libitum*. The animal room temperature was 19-21°C and the relative humidity was 52-83% with a 12 hour photoperiod.

Methods: Rats were ear-tagged and assigned to treatment groups:

Sex	Sacrifice Day	Groups/Ear tag Nos.				Total
		TG	KTG	NC	SC [c]	
M	0	501-503	513-515	525-527		9
	4	504-506	516-518	528-830		9
	7 [a]	507-509	519-521	531-533	537-539	12
	8 [b]	510-512	522-524	534-536		9
	Total	12	12	12	3	39
F	0	540-542	552-554	564-566		9
	4	543-545	555-557	567-569		9
	7 [a]	546-548	558-560	570-572	576-578	12
	8 [b]	549-551	561-563	573-575		9
	Total	12	12	12	3	39

M = Male; F = Female;
Group TG = *Beauveria bassiana* dosed rats that were housed together with the shelf control rats
Group SC = Shelf control rats that were housed together with the *Beauveria bassiana* dosed rats
Group KTG = Killed *Beauveria bassiana* (killed by autoclaving at 121°C for 15 minutes) dosed rats that were housed together with the naive control rats in a separate room
Group NC = Naive control rats that were housed in the same room with the killed *Beauveria bassiana* dosed rats
[a]Final enumeration endpoint
[b]Terminal sacrifice of remaining study animals
[c]SC animals sacrificed at the final enumeration time point, day 7

The rats were quarantined 7 days and fasted overnight prior to dosing. The test material (0.1 g) was added to 6 mL of ASTM 1 water, sonicated, and mixed until spores were completely in suspension. Half of the suspension was used to dose the TG group rats and the other half autoclaved at 121°C for 15 minutes was used to dose the KTG group rats. The total dose was 1 mL in a single challenge. TG group rats were dosed orally with approximately 5.1×10^7 cfu of *Beauveria bassiana* per animal in a 1 mL volume. Body weights were recorded on the day of dosing and on days 4 and 7. The test animals were observed for clinical signs twice daily during preliminary lethality and the pathogenicity assay. Three rats/sex/group from the TG, KTG, and NC groups were killed and necropsied on days 0, 4, and 7. The SC group rats were necropsied on day 7. The blood, brain, lungs, spleen, liver, kidneys, mesenteric lymph nodes, stomach and small intestines, and feces were removed for microbial enumeration and weight determination. Sensitivity of Detection of *Beauveria bassiana* was presented in MRID 45085202. Potato dextrose agar with 5 mg/L tetracycline (PDA/T) medium was used for stomach and small intestine and feces plating, the other tissues were plated on potato dextrose agar (PDA) medium. The microbial titer was determined by counting colonies on duplicates spread samples after incubation for 3-4 days at approximately 21°C. Initial concentration of the dosing suspensions or dilutions were verified. Statistic methods were presented for microbial enumeration, body weights, and organ weights.

II. RESULTS

Mortality: There were no deaths observed in any of the dosed or control groups.

Body Weights: All animals gained weight during the study.

Clinical Observations: No adverse signs were observed from any group.

Gross Necropsy: One female (No. 556) had a mottled kidney. No gross lesions were noted on any other rats at necropsy.

Organ Weights: No statistically significant effects on relative organ weights (lungs, spleen, liver, kidneys, brain, mesenteric lymph nodes, stomach and small intestines) were observed.

Infectivity results: No test organisms were detected in any sample (blood, brain, lungs, spleen, liver, kidneys, mesenteric lymph nodes, stomach and small intestines, or feces) from the KTG, NC, and SC group rats. *Beauveria bassiana* was only detected in the brain and stomach and small intestines on day 0 from the TG group rats. No test organisms were detected in any other tissue from TG group rats on days 0, 4, or 7. Results were shown below.

Recovery of viable *Beauveria bassiana* is shown in brain and stomach and small intestines in TG dosed males:

	Sacrifice Day and Mean Viable (CFU/g) Recovery		
	0	4	7
Brain	1.010 [a] 9.23 [b]	BDL [+]	BDL
Stomach and Small Intestines	8.204*[a] 1.60 x 10^{8} [b]	BDL [+]	BDL

Data taken from Table 9, p. 29-30, MRID 45144201.
[a]Mean of Log_{10} [(cfu/tissue) + 1]; N = 3
[b]Geometric mean of [(cfu/tissue) + 1]
BDL Below detection limit [<30 cfu/tissue]
*Significantly different from the naive control; $p \leq 0.05$.
[+]Significantly different from day 0; $p \leq 0.05$.

Recovery of viable *Beauveria bassiana* is shown in brain and stomach and small intestines in TG dosed females:

	Sacrifice Day and Mean Viable (CFU/g) Recovery		
	0	4	7
Brain	1.252 [a] 16.9 [b]	BDL [+]	BDL
Stomach and Small Intestines	7.863*[a] 7.29 x 10^{7} [b]	BDL [+]	BDL

Data taken from Table 10, p. 31-32, MRID 45144201.
[a]Mean of Log_{10} [(cfu/tissue) + 1]; N = 3
[b]Geometric mean of [(cfu/tissue) + 1]
BDL Below detection limit [<30 cfu/tissue]
*Significantly different from the naive control; $p \leq 0.05$.
[+] Significantly different from the day 0; $p \leq 0.05$.

III. DISCUSSION

The presented data showed no clinically significant signs in any rat. *Beauveria bassiana,* isolate 447 was not detected in the kidney, liver, lungs, spleen, mesenteric lymph nodes, feces, or blood samples and was cleared from the brain and stomach and small intestines by day 4. Necropsy studies showed no significant signs of abnormalities. Therefore, based on the presented/ submitted data, the test organism was not toxic, infective, or pathogenic to rats. The packet classification is **ACCEPTABLE.**